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Amino acid transport in *Lactobacillus helveticus*

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Abstract

Amino acid transport in *Lactobacillus helveticus* was analyzed. Strain specificity of amino acid transport was speculated between *L. helveticus* NCDO2712 and SBT2171. Glucose energized *L. helveticus* NCDO2712 actively transported and accumulated the essential and growth stimulating amino acids (leucine, isoleucine, valine, threonine, lysine, aspartic acid, glutamic acid, tyrosine, arginine, and histidine). Uptake of proline, phenylalanine and tryptophan was not observed. Transport studies in isolated membrane vesicles of *L. helveticus* fused with liposomes to reduce the passive proton permeability revealed that amino acids leucine, isoleucine, valine, threonine, and lysine are transported by a proton motive force coupled system. These results indicated that *L. helveticus* and *Lactococcus lactis* amino acid transport systems are similar. © 1998 Federation of European Microbiological Societies. Published by Elsevier Science B.V.

Keywords: Amino acid transport; *Lactobacillus helveticus*; Proton motive force

1. Introduction

Lactobacillus helveticus is a Gram-positive, facultatively anaerobic thermotrophic lactic acid bacterium. It is used industrially in dairy starter cultures for fermented milk, sour milk, and Swiss-type cheeses. *L. helveticus* was found to have the highest peptidase activities of 12 lactobacillus species studied [1]. This property makes *L. helveticus* an attractive potential candidate for application in accelerated cheese ripening.

Lactobacilli require many nutrients including several amino acids in the growth media [2]. *L. helveticus* can grow in milk which contains only small

quantities of free amino acids and possesses a proteolytic system to release amino acids from the milk protein, casein [3,4].

Recently, the proteinases and peptidases of *L. helveticus* have been studied at both the biochemical and genetic levels [4]. The proteolytic enzymes were found to be similar but not identical to those found in the best studied lactic acid bacterium, *Lactococcus lactis* [4].

Only very limited information is available about the lactobacilli amino acid and peptide transport systems. Strobel et al. [5] reported a proton motive force (pmf) dependence of amino acid transport in *L. casei*. Recently, Stucky et al. [6] cloned a gene encoding a branched amino acids transporter (*brnQ*) from *L. delbruckii* subsp. *lactis*. From *L. helveticus*, we have cloned and sequenced the gene encoding a proton motive force (pmf) coupled di- and

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tripeptide transporter [7]. In this report, we describe properties of essential and growth stimulating amino acid transport systems in *L. helveticus*.

2. Materials and methods

2.1. Bacteria

L. helveticus SBT2171 was kindly provided by Snow Brand European Research laboratory (Groningen, The Netherlands). *L. helveticus* NCDO2712 was divided from Institute of Food Research, Reading, UK. Cells were transferred to glucose- or lactose-MRX broth [1] and grown for 16 h at 37°C.

2.2. Amino acid transport assay in whole cells

Preculture (incubated for 16 h at 37°C) was inoculated in 0.5% glucose-MRX broth and incubated for 5 h at 37°C. *L. helveticus* cells were washed twice with 100 mM potassium phosphate (pH 6.5; KPi) and subsequently rapid frozen in liquid nitrogen. The cells were stored at –70°C before use. Amino acid transport was performed as follows. The cells (OD_{660nm} approximately 0.5) were resuspended and incubated for 2 min at 37°C in 100 mM 2-*N*-morpholinoethanesulfonic acid (Mes)/KOH (pH 6.5) in the presence of 0.75% glucose. Transport was started by the addition of [¹⁴C]-labelled amino acid. Samples of 100 µl were taken at certain time points, filtered over 0.45 µm cellulose acetate membranes, followed by washing twice with 2 ml of cold 0.1 M LiCl. The radioactivity on the filters was measured with a liquid scintillation counter.

2.3. Preparation of membrane vesicles

An overnight culture of *L. helveticus* was inoculated into lactose-MRX medium and incubated for 5 h at 37°C (OD_{660nm} approximately 0.5). The cells were washed three times with 100 mM KPi (pH 7.0) and resuspended in the same buffer. After addition of mutanolysin (1600 U/ml final concentration) and lysozyme (10 mg/ml final concentration), the mixture was incubated for 10 min at 37°C. The remaining procedure was performed basically by the method of Driessen et al. [8]. To lyse the cells, saturated

K₂SO₄ was added (1/3 v/v). The suspension was diluted 1.8-fold with 100 mM KPi (pH 7.0). DNase and RNase (100 µg/ml of each enzyme final concentration) were added and the solution further incubated for 20 min at 37°C. Potassium EDTA and Mg₂SO₄ were added at final concentrations of 15 mM and 2 mM, respectively. Membrane vesicles and cells were harvested by high speed centrifugation (48 200×*g* for 30 min) and resuspended in 50 mM KPi (pH 6.5). Unlysed cells were removed by low speed centrifugation (750×*g* for 60 min) and the supernatant was used as the membrane vesicle fraction.

2.4. Preparation of hybrid membrane vesicles

Phosphatidylethanolamine (PE) was obtained from SIGMA Chemicals and re-extracted as described by Driessen et al. [8]. Re-extracted PE was mixed with egg phosphatidylcholine (PC) in a ratio of 3:1. Liposomes were obtained by ten cycles sonication of intervals of 4 s and 56 s rest at four microns of amplitude at 4°C under a constant stream of nitrogen.

Hybrid membrane vesicles were prepared by mixing the membrane vesicles of *L. helveticus* in 20 mM KPi (pH 6.5) containing 100 mM potassium acetate with PE/PC liposomes in a ratio of 1 to 10 by freeze-thaw-sonication as described by Driessen et al. [8]. Hybrid membrane vesicles were stored in liquid nitrogen before use.

2.5. Energization of transport by artificial ion gradients

Transport in hybrid membrane vesicles was measured as described [9]. Briefly, hybrid membrane vesicles were collected by centrifugation, resuspended in 20 mM KPi containing 100 mM potassium acetate (pH 6.5) and incubated for 1 h on ice. The hybrid membrane vesicles were diluted to 1/100 with 120 mM sodium phosphate (NaPi; pH 6.5) containing 1 µM valinomycin and [¹⁴C]-labelled amino acid. At given time intervals, the mixture was filtered immediately through cellulose nitrate membrane (pore diameter 0.45 µm). The filters were washed once with 2 ml of cold 0.1 M LiCl and radioactivity was measured by liquid scintillation. The uptake of radiola-

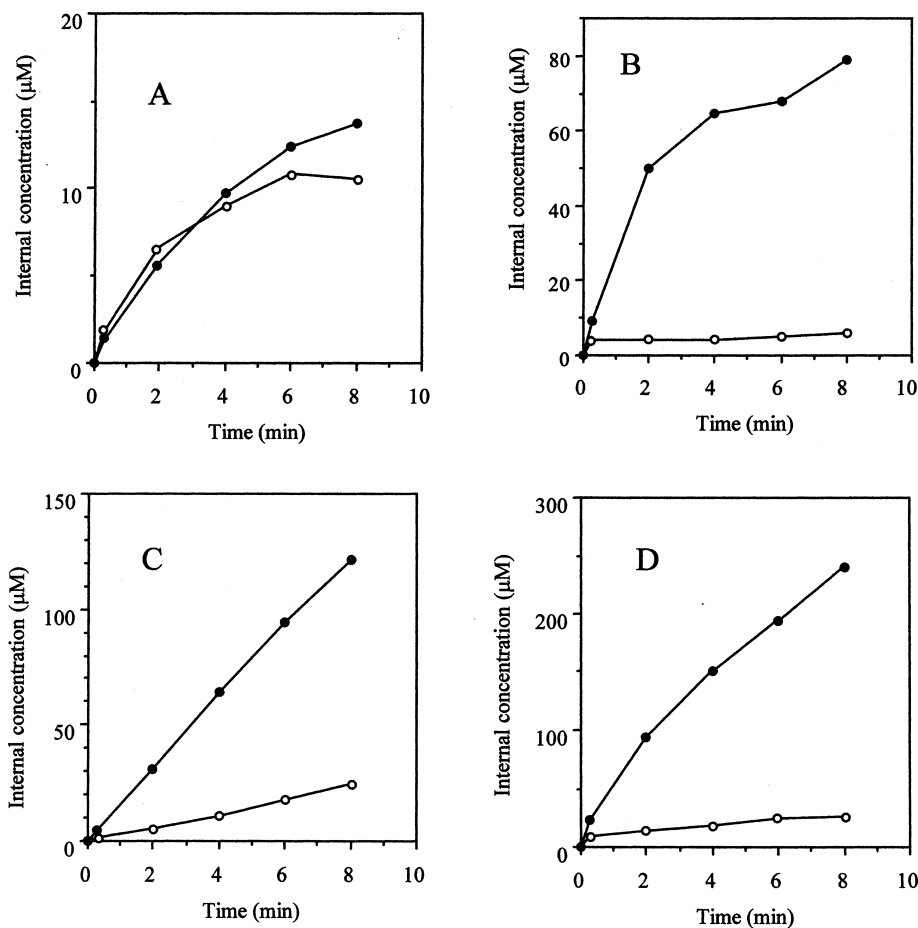


Fig. 1. Amino acid transport in glucose energized *L. helveticus*. Open circles, amino acids transported in industrially used *L. helveticus* SBT2171; closed circles, amino acids transported in *L. helveticus* NCDO2712. A: Alanine (external concentration was 3.25 mM); B: leucine (external concentration, 1.56 mM); C: aspartic acid (external concentration, 2.08 mM); D: histidine (external concentration, 14.4 mM).

belled methyl- β -D-thiogalactoside (TMG) was studied for comparisons.

2.6. Miscellaneous

Internal cell volume of *L. helveticus* was estimated as described by Poolman and Konings [10] using non-metabolizable sorbitol ($[^{14}\text{C}]$ -labelled) and $[^3\text{H}]$ -labelled water. All radiolabelled compounds were purchased from Amersham (Buckinghamshire, UK) or New England Nuclear (Dreieich, Germany). Protein was determined by the method of Lowry et al. [11] using bovine serum albumin as a standard.

3. Results and discussion

Fourteen amino acids have been reported to be essential or growth stimulating for *L. helveticus* [2]. The transport of all these amino acids except methionine was studied in whole cells. The transported amino acids may be metabolized during the uptake experiments. Therefore the uptake of amino acids was calculated from the accumulated radioactivity (Fig. 1). In Table 1, the accumulation levels after 8 min are given. These accumulation levels could be calculated after determining the internal volume of the cells. The internal cell volumes of *L. helveticus* SBT2171 and NCDO2712 were found to be

2.45 ± 0.44 and 2.50 ± 0.66 ml/mg protein (expressed as mean \pm standard deviation of four measurements), respectively.

In the presence of glucose as energy source, the uptake of leucine, aspartic acid and histidine were significantly higher in *L. helveticus* NCDO2712 than in the industrially used strain SBT2171 (Fig. 1). *L. helveticus* NCDO2712 accumulated ten amino acids (leucine, isoleucine, valine, threonine, lysine, aspartic acid, glutamic acid, tyrosine, arginine, and histidine) but not proline, phenylalanine, or tryptophan (Table 1).

To obtain information about the driving force for these amino acid transport processes, studies were performed in hybrid membrane vesicles in which an artificial proton motive force (pmf) of -120 mV was generated. Membrane vesicles from lactose-MRX grown *L. helveticus* were fused with liposomes prepared from *E. coli* phosphatidylethanolamine (PE) and egg phosphatidylcholine (PC) and transport studies were performed as described in Section 2. The transport of ten amino acids which were accumulated by the glucose energized cells was analyzed in these experiments. For comparison, the uptake of lactose analogue TMG was calculated.

The initial rates of uptake for ten amino acids are listed in Table 2. The initial uptake rate of TMG was

Table 1
Essential amino acids uptake in glucose energized *L. helveticus* NCDO2712 cells

	Ext. conc. ^a (μ M)	Apparent accumulation
Leu	1.56	44.2
Ile	1.55	31.2
Val	1.15	10.2
Thr	2.14	56.5
Lys	1.71	59.6
Asp	2.08	56.0
Glu	0.91	71.6
Tyr	1.00	20.4
Pro	3.48	0.79
Phe	92.5	0.70
Arg	1.52	6.5
His	14.4	15.0
Trp	34.2	1.1

^aExt. conc., external concentration of corresponding amino acid. Exponentially grown cells in MRX medium were used for uptake experiments. Amino acid accumulation expresses the ratio of the internal amino acid concentration over the external concentration.

Table 2

Proton motive force dependent transport of essential amino acids and TMG by *L. helveticus* hybrid membrane vesicles

	Ext. conc. ^a (μ M)	Initial rate (nmol/min/mg protein)
Val	0.90	0.33
Leu	0.81	0.24
Ile	0.80	0.16
Thr	1.11	0.19
Lys	0.89	0.17
Asp	1.20	n.d. ^b
Glu	1.48	n.d. ^b
His	1.49	n.d. ^b
Arg	0.16	n.d. ^b
Tyr	0.10	n.d. ^b
TMG	18.0	2.1

^aExt. conc., External concentration.

^bn.d., not detected.

2.1 nmol/min/mg. Pmf-driven uptake – by the hybrid membranes – of the amino acids leucine, isoleucine, valine, threonine, and lysine was observed, but not tyrosine, arginine, histidine, aspartic acid, and glutamic acid (Table 2). These results indicated that leucine, isoleucine, valine, threonine, and lysine are transported by a pmf-driven secondary transport system. The driving force of the other amino acids is not known.

These results differ from those obtained by Strobel et al. in *L. casei* [5]; they found at least five distinct amino acid transport systems which are involved in the uptake of glutamine, asparagine, glutamate/aspartate, phenylalanine, and branched chain amino acids. Valine, isoleucine, arginine, cysteine, tyrosine, and tryptophan are actively transported by glucose energized *L. casei*.

Amino acid transport systems of *L. lactis* have been studied in great detail by Poolman [12]. At least five secondary amino acid transporters (branched amino acids, alanine and threonine, serine and threonine, and lysine transporters) and at least three ATP coupled systems (glutamine and glutamate, and asparagine and aspartate systems) were mentioned.

It is of interest that the industrially used *L. helveticus* strain SBT2171, which is highly adapted to growth in milk, accumulates significantly less amino acids than the standard strain NCDO2712. The industrial strain SBT2171 can not grow in chemically defined medium with amino acids as sole nitrogen

sources (Sasaki et al., personal communication). Evidently, this strain has to obtain most of its essential amino acids from the uptake of peptides [7].

Our amino acid transport experiments indicate the presence of essential and growth stimulating amino acid transporters in *L. helveticus* [13]. Leucine, isoleucine, valine, threonine, and lysine are transported by secondary transport systems in *L. helveticus* and *L. lactis* [12]. Leucine, isoleucine, and valine are transported by a pmf coupled branched amino acid transporter (BrnQ) in *L. delbrückii* subsp. *bulgaricus* [6]. The amino acids aspartate, glutamate, histidine, arginine, and tyrosine are most likely transported by primary ATP-driven systems [13] as was found for aspartate and glutamate in *L. lactis* [12] and *L. casei* [5]. *L. lactis* transports tyrosine and histidine by pmf coupled transport systems [12]. In some lactic acid bacteria, histidine, tyrosine and arginine are transported by a special class of secondary transport systems, e.g. precursor/product antiporter systems [9]. These amino acids are metabolized internally by the enzymes histidine decarboxylase, tyrosine decarboxylase, and arginine deiminase, respectively. These enzymes could not be detected in *L. helveticus* (data not shown). Recently, Liu et al. [14] reported the presence of arginine deiminase in hetero-fermentative but not in homo-fermentative lactobacilli. Our results are consistent with this conclusion. For the third group of amino acids, proline, phenylalanine and tryptophan, it is far from clear how these amino acids are transported.

In conclusion, the results presented indicated that amino acids in *L. helveticus* are transported via similar systems to *L. lactis* and that during growth in milk, peptide transport systems may be as important in *L. helveticus* as they are in *L. lactis* [4].

Acknowledgments

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References

- [1] Sasaki, M., Bosman, B.W. and Tan, P.S.T. (1995) A comparative study on proteolytic activity in various *Lactobacillus* strains. *J. Dairy Res.* 62, 601–610.
- [2] Morishita, T., Deguchi, Y., Yajima, M., Sakurai, T. and Yura, T. (1981) Multiple nutritional requirements of lactobacilli: Genetic lesions affecting amino acid biosynthetic pathways. *J. Bacteriol.* 148, 64–71.
- [3] Poolman, B., Kunji, E.R.S., Hagting, A., Juillard, V. and Konings, W.N. (1995) The proteolytic pathway of *Lactococcus lactis*. *J. Appl. Bacteriol.* 79, 65S–75S.
- [4] Kunji, E.R.S., Smid, E.J., Plapp, R., Poolman, B. and Konings, W.N. (1996) The proteolytic systems of lactic acid bacteria. *Antonie van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* 70, 123–131.
- [5] Strobel, H.J., Russell, J.B., Driessen, A.J.M. and Konings, W.N. (1989) Transport of amino acids in *Lactobacillus casei* by proton-motive-force-dependent and non proton motive force dependent mechanisms. *J. Bacteriol.* 171, 280–284.
- [6] Stucky, K., Hagting, A., Klein, J.R., Matern, H., Henrich, B., Konings, W.N. and Plapp, R. (1995) Cloning and characterization of *brnQ*, a gene encoding a low-affinity, branched chain amino acid carrier in *Lactobacillus delbrückii* subsp. *lactis* DSM7290. *Mol. Gen. Genet.* 249, 682–690.
- [7] Nakajima, H., Hagting, A., Kunji, E.R.S., Poolman, B. and Konings, W.N. (1997) Cloning and functional expression in *Escherichia coli* of the gene specifying di-, tripeptide transport protein of *Lactobacillus helveticus*. *Appl. Environ. Microbiol.* 63, 2213–2217.
- [8] Driessen, A.J.M., de Vrij, W. and Konings, W.N. (1985) Incorporation of beef heart cytochrome c oxidase as a proton-motive force-generating mechanism in bacterial membrane vesicles. *Proc. Natl. Acad. Sci. USA* 82, 7555–7559.
- [9] Tolner, B., Ubbink-Kok, T., Poolman, B. and Konings, W.N. (1992) Characterization of the proton/glutamates symport protein of *Bacillus subtilis* and its functional expression in *Escherichia coli*. *J. Bacteriol.* 177, 2863–2869.
- [10] Poolman, B. and Konings, W.N. (1988) Relation of growth of *Streptococcus lactis* and *Streptococcus cremoris* to amino acid transport. *J. Bacteriol.* 170, 700–707.
- [11] Lowry, O.H., Farr, A.L. and Randall, R.J. (1951) Protein measurement with the Golin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- [12] Poolman, B. (1993) Energy transduction in lactic acid bacteria. *FEMS Microbiol. Rev.* 12, 125–148.
- [13] Konings, W.N., Poolman, B. and van Veen, H.W. (1994) Solute transport and energy transduction in bacteria. *Antonie van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* 5, 369–380.
- [14] Liu, S.-Q., Pritchard, G.G., Hardman, M.J. and Pilone, G.J. (1995) Occurrence of arginine deiminase pathway enzymes in arginine catabolism by wine lactic acid bacteria. *Appl. Environ. Microbiol.* 61, 310–316.